

[0017] C: Coupling of peptide to Tetanus toxoid:

[0018] Six ml of the reduced peptide was added to 0.3 ml of TT labeled with N-hydroxysuccinimidyl iodoacetate and 1 ml 5×HEPES buffer. After overnight incubation at 4° C., the conjugate was concentrated to about 1 ml using a MACROSEP™ 50 device, then desalted into HEPES buffer using P6 cartridges, concentrated again (MACROSEP™ 50), and, finally, filtered through a 0.45 micron Millex HV filter (Millipore). Evaluation of the protein content using the BioRad assay showed total protein content to be about 2.6 mg/ml.

[0019] Monoclonal Antibody Production:

[0020] A: Preparation of anticonsensus peptide monoclonal antibody:

[0021] Six BALB/c mice identified as numbers 8378-8383, were immunized with the consensus peptide-TT conjugate. On designated day 1, each mouse was injected subcutaneously with 25  $\mu$ g conjugate in 0.2 ml emulsified in 60% complete Freund's adjuvant. On day 23, a serum sample was obtained from each mouse. On day 35, all mice except # 8382 received a boost of 10  $\mu$ g consensus peptide conjugate in 0.2 ml 60% incomplete Freund's adjuvant. Mouse 8382 was given 10  $\mu$ g conjugate of the peptide in 0.1 ml phosphate-buffered saline (PBS).

[0022] On day 37, mouse 8382 was used for fusion (96-104). This fusion did not result in production of a monoclonal anti-consensus peptide.

[0023] On day 82, the mice received booster immunizations of 10  $\mu$ g consensus peptide conjugate in 0.2 ml emulsified in 60% incomplete Freund's adjuvant.

[0024] On day 85, the spleen from mouse #8383 was fused with FOX-NY myeloma wherein the myeloma population viability was 97.4%.  $1.36 \times 10^8$  spleen cells were fused with  $1.37 \times 10^7$  myeloma cells, using PEG (1400 molecular weight) as a fusogen. The hybridomas was assigned culture number 96-109.

[0025] Hybridomas were planted into 8 96-well tissue culture dishes with 100  $\mu$ l/well in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 10% Hybridoma Serum Free Media (SFM), 100  $\mu$ M hypoxanthine and 16  $\mu$ M thymidine (the hypoxanthine and thymidine combination being referred to herein as HT). Eight wells were also planted with FOX-NY myeloma cells only (no hybridomas) as a control. All samples were incubated at 37° C. in a humidified atmosphere of 5% CO<sub>2</sub> in air. After 24 hours, all wells received 100  $\mu$ l RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 10% hybridoma SFM, 200  $\mu$ M hypoxanthine, 0.8  $\mu$ M aminopterin and 32  $\mu$ M thymidine. (The hypoxanthine, aminopterin and thymidine combination being referred to herein as HAT.)

[0026] Approximately 96 hours after the fusion, the FOX-NY myelomas in control wells appeared to be dead. Many other wells contained growing colonies of hybridomas seven days after fusion. The growing cells were fed by addition of RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum, 10% hybridoma SFM and HT. Four days thereafter, the supernatants were tested for the presence of anti-consensus peptide antibodies.

[0027] For analysis of peptide binding, an ELISA was used. Nunc MAXISORP™ stripwells were coated overnight

at room temperature with 100  $\mu$ l/well of consensus peptide at 1  $\mu$ g/ml PBS. The wells were then washed four times with PBS containing 0.05% TWEEN-20™ (PBS-T) to remove unbound material. Each well then received 50  $\mu$ l of PBS-T. Fifty  $\mu$ l of supernatant was then transferred from the cell culture plate to the corresponding wells of the immunoassay dish. Prior to the transfer of the cell culture, wells were screened microscopically to identify wells without hybridomas. One such well from each plate was used as a background control by substituting PBS-T or medium for the culture supernatant. The plates were then sealed and incubated for 30 to 60 minutes at room temperature in the dark in a draft-free environment. The wells were thereafter washed four times with PBS-T to remove unbound material. Each well then received 95  $\mu$ l of sheep anti-mouse IgG-HRP (horse radish peroxidase), diluted 1:10000 in PBS-T. Following a 30 minute incubation, the wells were again washed and each well received 100  $\mu$ l of tetramethylbenzidine (TMB) substrate solution. The plates were then incubated in the dark for 15 minutes at room temperature, after which the reactions were stopped by addition of 80  $\mu$ l of TMB Stop Solution. The absorbance of each well was determined at 450 nm using a Molecular Devices microplate reader.

[0028] Absorbance values for 32 of the supernatants from wells with growing hybridomas was greater than 0.200 units. Of these, only two wells, designated CA8 (1.743) and FE8 (1.092) had absorbance values of greater than 1.000. All thirty-two cultures were expanded by transfer into 24-well culture dishes and grown on RPMI 1640 with 10% FBS. Upon retest, only colony FE8 continued to produce antibodies reactive with the consensus peptide. This culture was expanded to growth in a T75 culture flask and samples were cryopreserved.

[0029] The isotype of the antibody secreted by 96-109FE8 was determined using a Zymed isotype kit. The results indicated that the antibody was an IgM with a kappa light chain. The 96-109FE8 culture was cloned into 96-well culture dishes by diluting the cells to a concentration of 4.5-5 cells/ml in RPMI 1640 with 20% FBS and 10% hybridoma SFM. Each well received 200  $\mu$ l of the cell suspension. Each well was checked for the presence of a single focus of growing hybridomas. The supernatants from each such well were tested for binding of the antibody to the consensus peptide epitope. All of the supernatants were active, suggesting that all of the surviving cells in the original culture were secretors of the antibody of interest, and that the genotype was stable. One clone, designated 96-109FE8 1h11, was expanded, cryopreserved and used in the production of ascites.

[0030] Testing of hybridoma tissue culture supernatant for agglutinating activity:

[0031] Bacterial culture: ETEC strains bearing the colonization factors CFA/I, CS 1, CS2 and CS4 were grown overnight at 37° C. on colonization factor antigen agar (10 gm Casamino acids, 1% (Difco Laboratories, Detroit, Mich.); 1.5 gm yeast extract (Difco), 0.15%; 0.1 gm MgSO<sub>4</sub> 7H<sub>2</sub>O, 0.005% (Sigma, St. Louis); 0.008 gm MnCl<sub>2</sub>, 0.0005% MnCl<sub>2</sub> (Sigma); 20 gm agar (Difco);, q.s. to 1 liter with deionized water). Those ETEC strains bearing the colonization factors CS 17 and PCF 0166 are also grown on colonization factor antigen agar, which was also supplemented with 0.15% bile salts (bile salts #3, Difco). Bacteria